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Efficacy of Delayed Treatment with ST-246 Given Orally against Systemic Orthopoxvirus Infections in Mice▽

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ST-246 was evaluated for activity against cowpox virus (CV), vaccinia virus (VV), and ectromelia virus (ECTV) and had an in vitro 50% effective concentration (EC_{50}) of 0.48 μM against CV, 0.05 μM against VV, and 0.07 μM against ECTV. The selectivity indices were >208 and >2,000 for CV and VV, respectively. The in vitro antiviral activity of ST-246 was significantly greater than that of cidofovir, which had an EC_{50} of 41.1 μM against CV and 29.2 μM against VV, with selectivity indices of >7 and >10, respectively. ST-246 administered once daily by oral gavage to mice infected intranasally with CV beginning 4 h or delayed until 72 h postinoculation was highly effective when given for a 14-day duration using 100, 30, or 10 mg/kg of body weight. When 100 mg/kg of ST-246 was administered to VV-infected mice, a duration of 5 days was sufficient to significantly reduce mortality even when treatment was delayed 24 h postinoculation. Viral replication in liver, spleen, and kidney, but not lung, of CV- or VV-infected mice was reduced by ST-246 compared to levels for vehicle-treated mice. When 100 mg/kg of ST-246 was given once daily to mice infected by the intranasal route with ECTV, treatment for 10 days prevented mortality even when treatment was delayed up to 72 h after viral inoculation. Viral replication in target organs of ECTV-infected mice was also reduced.

National preparedness for bioterrorist events includes the development of rapid detection techniques, improved vaccination strategies, and antimicrobial chemotherapeutics with differing modes of action or targets. Preparing for a weaponized variola virus release is one component necessary for national security, and the development of highly effective, nontoxic antiviral agents that have proven efficacy when given postexposure is essential. Although cidofovir (CDV) has been approved under investigational new drug application for treatment of smallpox or complications of vaccination, its use would be limited since it is not orally bioavailable and produces nephrotoxicity.

Previous evaluation of ST-246 for activity against orthopoxviruses has shown both in vitro and in vivo efficacies (13). When evaluated in vitro against vaccinia virus (VV), cowpox virus (CV), ectromelia virus (ECTV), monkeypox virus, camelpox virus, and variola virus, ST-246 inhibited virus replication by 50% at a concentration (50% effective concentration [EC_{50}]) of ≤0.07 μM. In animal models using lethal infections with ECTV or VV, ST-246 was reported to be nontoxic and effective against mortality when given orally twice daily at 50 mg/kg of body weight for 14 days beginning before or shortly after infection. ST-246 was also evaluated in the nonlethal mouse tail lesion model by use of intravenous VV to mimic the primary viremic phase of viral infection and systemic lesional disease. When ST-246 was administered by oral gavage at 15 or 50 mg/kg twice daily for 5 days, the tail lesions were significantly reduced (13).

The current studies expand upon the previous in vitro and in vivo findings and further define parameters regarding time intervals postexposure and minimum length of time necessary for efficacy, as well as dose responses against CV in addition to VV and ECTV. Efficacy using delayed initiation of treatment after viral inoculation is an important determining factor for the selection of antiviral compounds to pursue in development. The results of these additional animal models for CV, VV, and ECTV using delayed treatment add valuable insight into the utility of this particular compound for use in treatment of orthopoxvirus infections in humans.

MATERIALS AND METHODS

Cells, viruses, and in vitro assays. CV, strain Brighton, and VV, strain Copenhagen, were kindly provided by John W. Huggins (Department of Viral Therapeutics, Virology Division, U.S. Army Medical Research Institute for Infectious Diseases, Frederick, MD). VV, strain WR, was obtained from the American Type Culture Collection (ATCC, Manassas, VA). Stock virus pools were propagated in Vero cells also obtained from ATCC. In vitro susceptibility assays with human foreskin fibroblasts (HFF) were performed as described previously (4). Briefly, to determine efficacy, HFF seeded in six-well plates 2 days prior to use were infected with either VV, strain Copenhagen, or CV by the addition of 20 to 30 PFU per well. After a 1-h incubation period, various concentrations of drug were added to triplicate wells and plates incubated at 37°C for 3 days. After incubation, cell monolayers were stained with neutral red for approximately 5 to 6 h, viral plaques were enumerated, and the EC_{50} was determined. For toxicity, the 50% cytotoxic concentration (CC_{50}) was evaluated using HFF seeded in 96-well plates, incubated with various concentrations of drug for 7 days at 37°C, and the cell monolayers were then stained with neutral red.

Ectromelia virus, strain Moscow (ECTV-MOS), was derived from a plaque-purified isolate (ATCC, VR-1374) as previously described (2). In vitro susceptibility studies using ST-246 against ECTV in Vero cells have been reported previously (13).

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TABLE 1. Cytotoxicity and efficacy of ST-246 against VV or CV in HFF cells

Compound	Vaccinia Copenhagen			Vaccinia WR		Cowpox Brighton	
	CC_{50} (μM)[a]	EC_{50} (μM)[a]	SI[b]	EC_{50} (μM)[a]	SI[b]	EC_{50} (μM)[a]	SI[b]
ST-246	>100 ± 0	0.05 ± 0.02	>2,000	0.1 ± 0.05	>1,000	0.48 ± 0.01	>208
CDV	>317 ± 0	29.2 ± 14	>10.9	33 ± 13	>9.6	41.1 ± 4.2	>7.7

[a] Values are the means of two or more assays ± standard deviations.
[b] Selectivity indices (SI) were determined by the following formula: CC_{50}/EC_{50}.

Antiviral compounds. CDV (Vistide; Gilead Sciences, Inc., Foster City, CA) was diluted in sterile saline to yield the desired dosages within a 0.1-ml volume. It was administered intraperitoneally (i.p.) as a single dose or once daily for 5 days as the positive control, depending on the protocol. ST-246 was synthesized and supplied by SIGA Technologies (Corvallis, OR) through NIAID, NIH. It was suspended in aqueous 0.75% methylcellulose (Sigma, St. Louis, MO) containing 1% Tween 80 (Sigma) to yield the desired dosage of 100, 50, 30, or 10 mg/kg within a 0.2-ml volume for VV or CV infections. For ECTV infections, ST-246 was given in a 0.1- or 0.2-ml volume daily at a dosage of 100 or 20 mg/kg. ST-246 was administered by oral gavage once daily for 5 to 14 days beginning 4, 24, 48, or 72 h after viral inoculation.

Mice. Female BALB/c mice, 3 to 4 weeks of age, were obtained from Charles River Laboratories (CRL, Raleigh, NC). BALB/c mice were utilized in models of systemic infection of VV or CV. Mice were housed in microisolator cages and utilized at 15 mice per group. Mice were obtained, housed, utilized, and euthanized according to policies of the USDA and the Association for Assessment and Accreditation of Laboratory Animal Care, International. All animal procedures were approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee prior to the initiation of studies.

Male or female A/NCr mice were obtained from the National Cancer Institute (Frederick, MD) and used at 6 to 11 weeks of age for ECTV models. Mice were utilized at four to eight mice per treatment group for efficacy or pathogenesis studies using ECTV-MOS. All animal procedures were approved by Saint Louis University School of Medicine's Institutional Animal Care and Use Committee prior to initiation of studies.

Experimental inoculations. Systemic CV or VV infections were initiated by intranasal (i.n.) inoculation of BALB/c mice as described previously (7). Mice were anesthetized using ketamine-xylazine and infected with an approximate 90% lethal dose of CV, strain BR (9×10^5 PFU/mouse), or VV, strain WR (1×10^4 PFU/mouse), by use of a micropipettor and a total volume of 40 μl per

TABLE 2. Effect of duration of treatment with ST-246 on the mortality of BALB/c mice inoculated intranasally with cowpox virus, strain BR, or vaccinia virus, strain WR

Treatment[a]	Cowpox virus, strain BR				Vaccinia virus, strain WR			
	No. of mice that died/ total no. of mice (%)	*P* value	MDD[b]	*P* value	No. of mice that died/ total no. of mice (%)	*P* value	MDD	*P* value
5-day duration, +4 h								
Vehicle	15/15 (100)		9.1		15/15 (100)		6.1	
ST-246 100 mg/kg	13/15 (87)	NS[c]	11.6	0.001	2/15 (13)	<0.001	3.0	<0.05
5-day duration, +24 h								
Vehicle	15/15 (100)		8.6		15/15 (100)		6.3	
ST-246 100 mg/kg	11/15 (73)	NS	12.4	<0.001	1/15 (7)	<0.001	3.0	0.08
CDV 15 mg/kg	0/15 (0)	<0.001			1/15 (7)	<0.001	15.0	0.08
7-day duration, +4 h								
Vehicle	15/15 (100)		8.2		15/15 (100)		5.7	
ST-246 100 mg/kg	1/15 (7)	<0.001	5.0	0.08	3/15 (20)	<0.001	6.3	NS
7-day duration, +24 h								
Vehicle	15/15 (100)		8.5		15/15 (100)		6.3	
ST-246 100 mg/kg	6/15 (40)	0.001	9.3	NS	1/15 (7)	<0.001	11.0	0.09
10-day duration, +4 h								
Vehicle	15/15 (100)		8.3		15/15 (100)		6.1	
ST-246 100 mg/kg	4/15 (27)	<0.001	8.0	NS	5/15 (33)	<0.001	10.6	0.06
10-day duration, +24 h								
Vehicle	15/15 (100)		7.9		15/15 (100)		6.1	
ST-246 100 mg/kg	6/15 (40)	0.001	13.2	<0.01	0/15 (0)	<0.001		
14-day duration, +4 h								
Vehicle	14/15 (93)		9.1		15/15 (100)		5.6	
ST-246 100 mg/kg	1/15 (7)	<0.001	3.0	0.09	3/15 (20)	<0.001	5.3	0.05
14-day duration, +24 h								
Vehicle	15/15 (100)		8.5		15/15 (100)		6.7	
ST-246 100 mg/kg	0/15 (0)	<0.001			1/15 (7)	<0.001	3.0	0.09

[a] ST-246 was supplied by SIGA, prepared in 0.75% methyl cellulose and 1% Tween 80, and delivered orally in 0.2-ml doses. CDV was prepared in sterile saline and given i.p. in 0.1-ml doses. Animals were treated once daily for 5, 7, 10, or 14 days beginning 4 or 24 h after viral inoculation (+4 h or +24 h, respectively).
[b] MDD, mean day of death.
[c] NS, not significant when compared to the appropriate vehicle control.

TABLE 3. Dose effect of delayed treatment with ST-246 on the mortality of BALB/c mice inoculated intranasally with cowpox virus

Treatment[a]	No. of mice that died/total no. of mice (%)	*P* value	MDD[b]	*P* value
4 h postinoculation				
Vehicle	15/15 (100)		9.0	
CDV 15 mg/kg	0/15 (0)	<0.001		
ST-246				
100 mg/kg	1/9 (11)	<0.001	10.0	NS
30 mg/kg	5/10 (50)	0.01	10.2	NS
10 mg/kg	11/12 (92)	NS[c]	12.2	<0.01
24 h postinoculation				
Vehicle	15/15 (100)		8.3	
CDV 15 mg/kg	0/15 (0)	<0.001		
ST-246				
100 mg/kg	4/15 (27)	<0.001	8.0	NS
30 mg/kg	6/15 (40)	0.001	10.5	NS
10 mg/kg	11/15 (73)	NS	14.3	<0.001
48 h postinoculation				
Vehicle	15/15 (100)		8.6	
CDV 15 mg/kg	0/15 (0)	<0.001		
ST-246				
100 mg/kg	1/15 (7)	<0.001	17.0	0.08
30 mg/kg	3/15 (20)	<0.001	14.3	NS
10 mg/kg	2/15 (13)	<0.001	11.0	NS
72 h postinoculation				
Vehicle	15/15 (100)		8.6	
CDV 15 mg/kg	0/15 (0)	<0.001		
ST-246				
100 mg/kg	6/15 (40)	0.001	16.8	<0.05
30 mg/kg	6/15 (40)	0.001	12.2	<0.05
10 mg/kg	7/15 (47)	<0.01	13.9	0.001

[a] ST-246 was supplied by SIGA, prepared in a vehicle of 0.75% methylcellulose with 1% Tween 80, and delivered orally in 0.2-ml doses. CDV was prepared in sterile saline and given i.p. in 0.1-ml doses. Animals were treated once daily for 14 days beginning 4, 24, 48, or 72 h after viral inoculation.

[b] MDD, mean day of death.

[c] NS, not significant when compared to the appropriate vehicle control.

animal. To determine the extent of viral replication in tissues, three animals from each treated and untreated group were euthanized on days 1, 3, 5, 7, and 10. Lung, liver, spleen, and kidney samples were removed aseptically, weighed, homogenized in minimal essential medium with 10% fetal bovine serum (10%, wt/vol) and frozen at −70°C until assayed for virus. Samples were thawed and assayed on Vero cells by use of an agarose-containing plaque assay to determine CV or VV titers (7). Briefly, samples of organ homogenates were diluted serially and a 0.2-ml volume was placed into 2 of 12 wells of Vero cell monolayers and incubated for 1 h. A 0.5% agar (SeaKem ME agarose; FMC BioProducts, Rockland, ME) in minimal essential medium solution was added to each well, and the cultures were incubated for 3 days. Cultures were stained with neutral red (Sigma) for approximately 6 h prior to enumeration of viral plaques.

Systemic infections of ECTV were initiated by i.n. inoculation of anesthetized A/NCr mice with approximately 11 50% lethal doses (LD_{50}) of ECTV in a total volume of 5 or 10 μl unless otherwise indicated. Mice were monitored daily after infection for clinical signs. Body weights were recorded three times weekly. To determine the extent of viral replication in tissues, five animals from selected treated and untreated groups were euthanized on days 4, 6, and 8 after viral inoculation. Lung, liver, spleen, esophagus, trachea, and nasal wash samples were obtained aseptically for virus quantitation as described previously (13).

Statistical evaluation. Groups of mice treated with antivirals were compared to vehicle-treated groups for statistical evaluation. Mortality rates were analyzed by Fisher's exact test. The mean day of death data were analyzed by the Mann-Whitney U rank sum test, which compares the median values nonparametrically. A *P* value of 0.05 or less was considered significant.

RESULTS

In vitro activity. ST-246 had an in vitro EC_{50} of 0.48 μM against CV and 0.05 μM against VV, with selectivity indices of >208 and >2,000, respectively (Table 1). This increased sensitivity of VV over CV is consistent with our past screening experience, but we have not addressed this further. These in vitro activities exceeded those of CDV by 27- and 183-fold, respectively. As published previously, ST-246 also had an in vitro EC_{50} of 0.07 μM against ECTV (13). The CC_{50} value of >100 μM for ST-246 in HFF indicated a low level of cytotoxicity.

Effect of ST-246 treatment on mortality and pathogenesis of CV and VV infections in mice. ST-246 was administered orally to CV-infected mice for various durations of 5, 7, 10, or 14 days using 100 mg/kg once daily beginning either 4 or 24 h after CV inoculation. Mortality was not reduced significantly using only 5 days of treatment, but a statistically significant increase in the mean day of death was achieved when treatments began either 4 or 24 h after virus inoculation (Table 2) ($P \leq 0.001$). All dosing regimens of 7 or more days in duration resulted in statistically significant decreases in mortality ($P \leq 0.001$) when ST-246 was given once daily at the 100-mg/kg dose. CDV was administered i.p. at 15 mg/kg for 5 days beginning 24 h after viral inoculation and was effective in preventing mortality ($P < 0.001$).

To determine the activity of ST-246 given at lower dosages and at later time points in the infection, CV-infected mice were given ST-246 at 100, 30, or 10 mg/kg beginning 4, 24, 48, or 72 h postinoculation. Treatments were given once daily for a duration of 14 days. When ST-246 was given at 100 mg/kg, mortality was significantly reduced (Table 3) ($P \leq 0.001$) if treatments were initiated at 4, 24, 48, or 72 h after viral inoculation. If ST-246 was given at 30 mg/kg, mortality was significantly reduced if treatments were initiated at 4 h postinfection ($P \leq 0.01$) and at 24, 48, or 72 h ($P \leq 0.001$). When ST-246 was given at 10 mg/kg, mortality was not reduced when treatments were initiated at 4 or 24 h postinfection; however, ST-246 beginning at 48 or 72 h did reduce mortality to statistically significant levels ($P \leq 0.01$). CDV was administered i.p. at 15 mg/kg for 5 days beginning 24, 48, or 72 h after viral inoculation and was effective in preventing mortality ($P < 0.001$).

ST-246 was administered orally to VV-infected mice for various durations of 5, 7, 10, or 14 days at 100 mg/kg beginning either 4 or 24 h after VV inoculation. Mortality was significantly reduced with 5, 7, 10, or 14 days of treatment (Table 2) ($P \leq 0.001$). CDV was administered i.p. at 15 mg/kg for 5 days beginning 24 h after viral inoculation and was effective in preventing mortality ($P < 0.001$).

To determine the effect of ST-246 treatment on viral replication in target organs, ST-246 was administered orally to CV- or VV-infected mice for 9 days at 50 mg/kg beginning 24 h postinfection. Mortality was significantly reduced (data not shown) ($P \leq 0.001$) and virus titers were reduced in target organs, in most cases below the levels detected in vehicle-treated mice (Fig. 1 and 2). CDV was administered i.p. at 15 mg/kg for 5 days beginning 24 h postinfection and was effective in preventing mortality (data not shown) ($P < 0.001$) and in reducing viral replication in liver, spleen, and kidney. Lung titers are not usually reduced by antiviral therapy in these models, even CDV, since the models are initiated by rather large inocula of virus administered intranasally in comparison

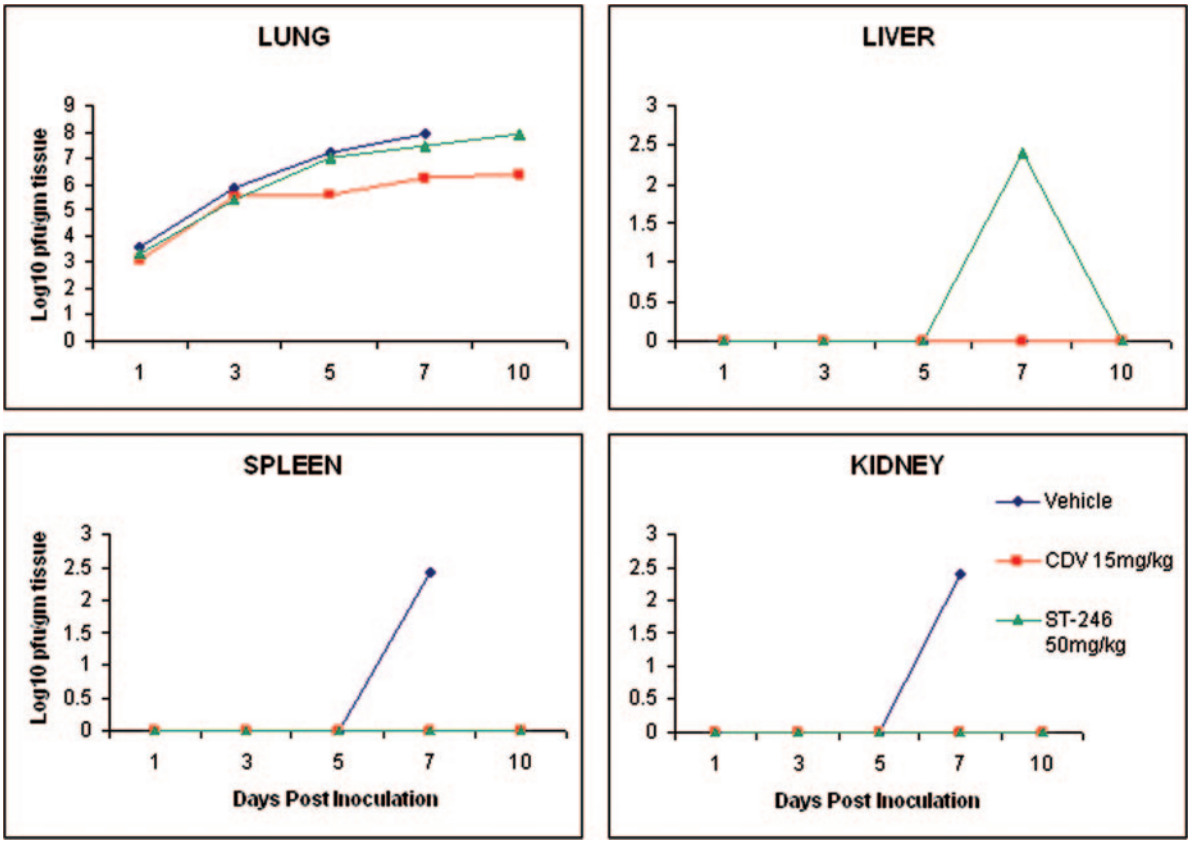

FIG. 1. Effects of once-daily oral dosing with 50 mg/kg ST-246 using a 9-day duration in BALB/c mice infected intranasally with cowpox virus, strain BR. Points indicate the values obtained from three pooled organ samples per time point. The limit of detection is 100 PFU/g.

to the ECTV models using very few PFU/mouse to initiate lethal infections. A consistent and important finding in these studies is the time of systemic spread to target organs of liver, spleen, or kidney. With CV-infected mice, target organs are positive for virus only on days 5 to 7 or later, whereas VV-infected mice have virus detected on days 3 to 5 postinoculation. Detection of virus in target organs of liver, spleen, or kidney is predictive of mortality in these models.

Effect of ST-246 treatment on mortality and pathogenesis of ECTV infections in mice. ST-246 was administered orally to ECTV-infected mice for 10 days using 100 mg/kg beginning 0, 24, 48, or 72 h postinfection. Mortality was blocked (Table 4) ($P \leq 0.001$) for all dosing regimens using ST-246, and body weights were not significantly different from those of uninfected animals (data not shown). CDV was administered i.p. at 100 mg/kg as a single dose at 24, 48, or 72 h after viral inoculation and was effective in preventing mortality ($P < 0.001$) at all time points. When escalating infectious challenges were utilized, ST-246 given twice daily for 14 days at 100 mg/kg beginning 4 h postchallenge maintained efficacy by eliminating mortality in A/NCr mice (Table 5). CDV, included as a positive control at 100 mg/kg given as a single dose, was also effective.

When ST-246 was given twice daily by gavage to ECTV-infected mice at a dose of 100 mg/kg for a duration of 14 days, mortality was reduced and virus replication was reduced for liver, lung, spleen, esophagus, trachea, and nasal wash samples compared to results with vehicle-treated mice. Viral quantities derived from day 4, 6, and 8 samples of lung, liver, or spleen are shown in Fig. 3. No virus was detected in ST-246-treated mice for liver, lung, spleen, esophagus, or trachea samples on days 4, 6, or 8 after viral inoculation. Some ECTV, however, was recovered in nasal wash samples of ST-246-treated mice on days 6 and 8 (data not shown). CDV also reduced viral load below the limit of detection for each of the animals given a single injection of 100 mg/kg (data not shown).

DISCUSSION

ST-246 demonstrated excellent in vitro activity against VV, CV, and ECTV with little in vitro toxicity. ST-246 was more active than CDV in these in vitro assays (5).

ST-246 proved to be highly effective in three different animal models of systemic orthopoxvirus disease even when treatment was delayed up to 72 h after viral inoculation and dosing was reduced to once daily. Uninfected animals treated with ST-246 using daily oral dosages of 100 mg/kg for extended times of 2 weeks showed no evidence of toxicity or weight loss. Regarding the duration of dosing on efficacy, the models vary rather strikingly when consideration is given to length of survival following infection of the vehicle-treated animals. When VV, strain WR, or VV, strain IHD, is administered intranasally, the

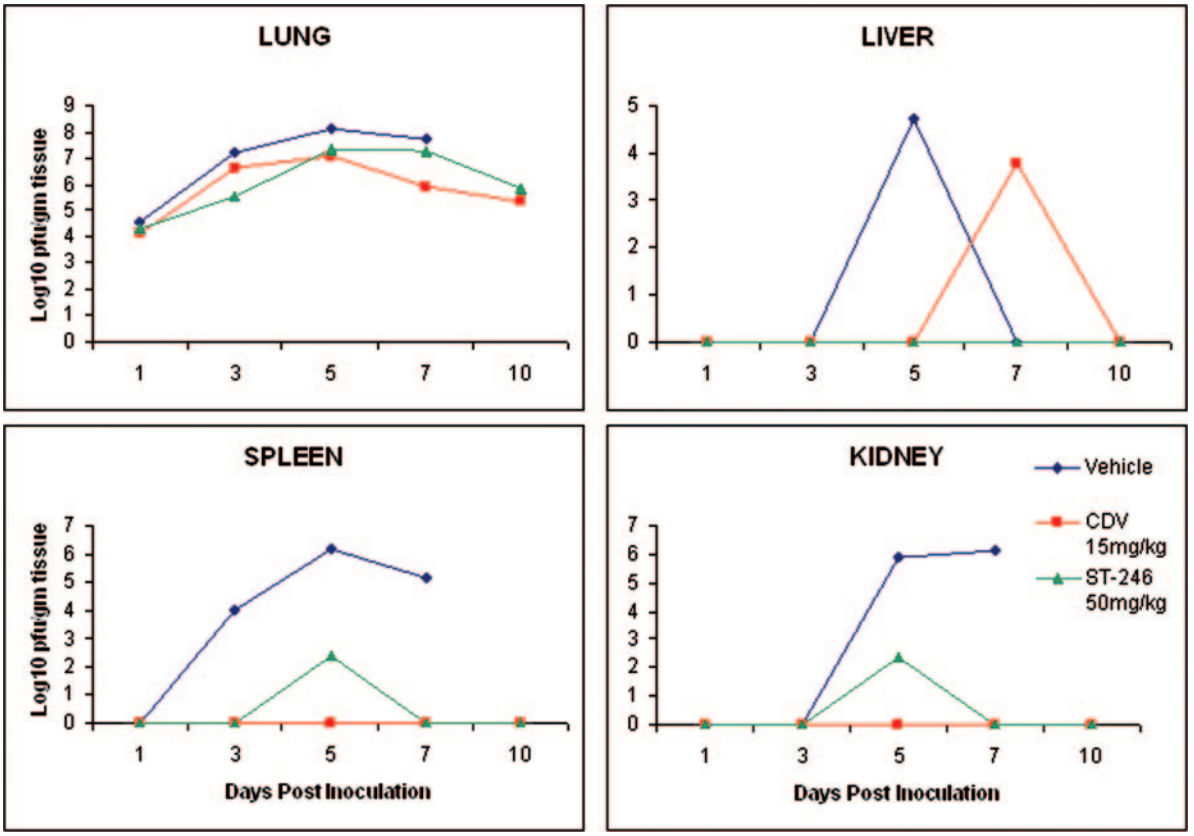

FIG. 2. Effects of once-daily oral dosing with 50 mg/kg ST-246 using a 9-day duration in BALB/c mice infected intranasally with vaccinia virus, strain WR. Points indicate the values obtained from three pooled organ samples per time point. The limit of detection is 100 PFU/g.

TABLE 4. Effect of delayed treatment with ST-246 on the mortality of A/NCr mice inoculated intranasally with ECTV

Treatment[a]	No. of mice that died/total no. of mice (%)	*P* value	MDD[c]	*P* value
Uninfected/not treated	0/8 (0)			
+0 h				
Mock infected plus vehicle	0/8 (0)			
Infected plus vehicle[b]	8/8 (100)		8.6	
ST-246 100 mg/kg	0/8 (0)	<0.001		
CDV 100 mg/kg	0/8 (0)	<0.001		
+24 h				
ST-246 100 mg/kg	0/8 (0)	<0.001		
CDV 100 mg/kg	0/8 (0)	<0.001		
+48 h				
ST-246 100 mg/kg	0/8 (0)	<0.001		
CDV 100 mg/kg	0/8 (0)	<0.001		
+72 h				
ST-246 100 mg/kg	0/8 (0)	<0.001		
CDV 100 mg/kg	0/8 (0)	<0.001		

[a] ST-246 was supplied by SIGA, prepared in 0.75% methyl cellulose and 1% Tween 80, and delivered orally in 0.2-ml doses. Animals were treated once daily for 10 days beginning 0, 24, 48, or 72 h after viral inoculation (+0 h, +24 h, +48 h, and +72 h, respectively). CDV was prepared in sterile saline and given only once as an i.p. injection using a 0.1-ml volume.
[b] Mice were inoculated i.n. with 10 μl of ECTV-MOS (3.3 PFU, ~11× LD_{50}).
[c] MDD, mean day of death.

TABLE 5. Effect of twice-daily treatment with ST-246 on mortality of A/NCr mice inoculated intranasally with increasing doses of ECTV

Treatment[a]	No. of mice that died/total no. of mice (%)	*P* value	MDD[c]	*P* value
Uninfected/not treated	0/10 (0)			
Vehicle[b]				
4.1 PFU	1/5 (20)		10	
41 PFU	5/5 (100)		10.4 ± 4.3	
410 PFU	5/5 (100)		7.2 ± 0.4	
4,100 PFU	5/5 (100)		7.0 ± 0	
CDV 100 mg/kg				
4.1 PFU	0/5 (0)	NS[d]		
41 PFU	2/5 (40)	NS	24 ± 0	NS
410 PFU	0/5 (0)	<0.01		
4,100 PFU	0/5 (0)	<0.01		
ST-246 100 mg/kg				
4.1 PFU	0/5 (0)	NS		
41 PFU	0/5 (0)	<0.01		
410 PFU	0/5 (0)	<0.01		
4,100 PFU	0/5 (0)	<0.01		

[a] ST-246 was supplied by SIGA, prepared in 0.75% methyl cellulose and 1% Tween 80, and delivered by oral gavage 4 h postchallenge, twice daily in 0.1-ml doses for 14 days. CDV was prepared in sterile saline and given once as an i.p. injection using a 0.1-ml volume.
[b] Mice were inoculated i.n. with 5 μl of ECTV-MOS containing the indicated doses of virus (LD_{50} of approximately 0.6 PFU/mouse following the i.n. route).
[c] MDD, mean day of death.
[d] NS, not significant when compared to the appropriate vehicle control.

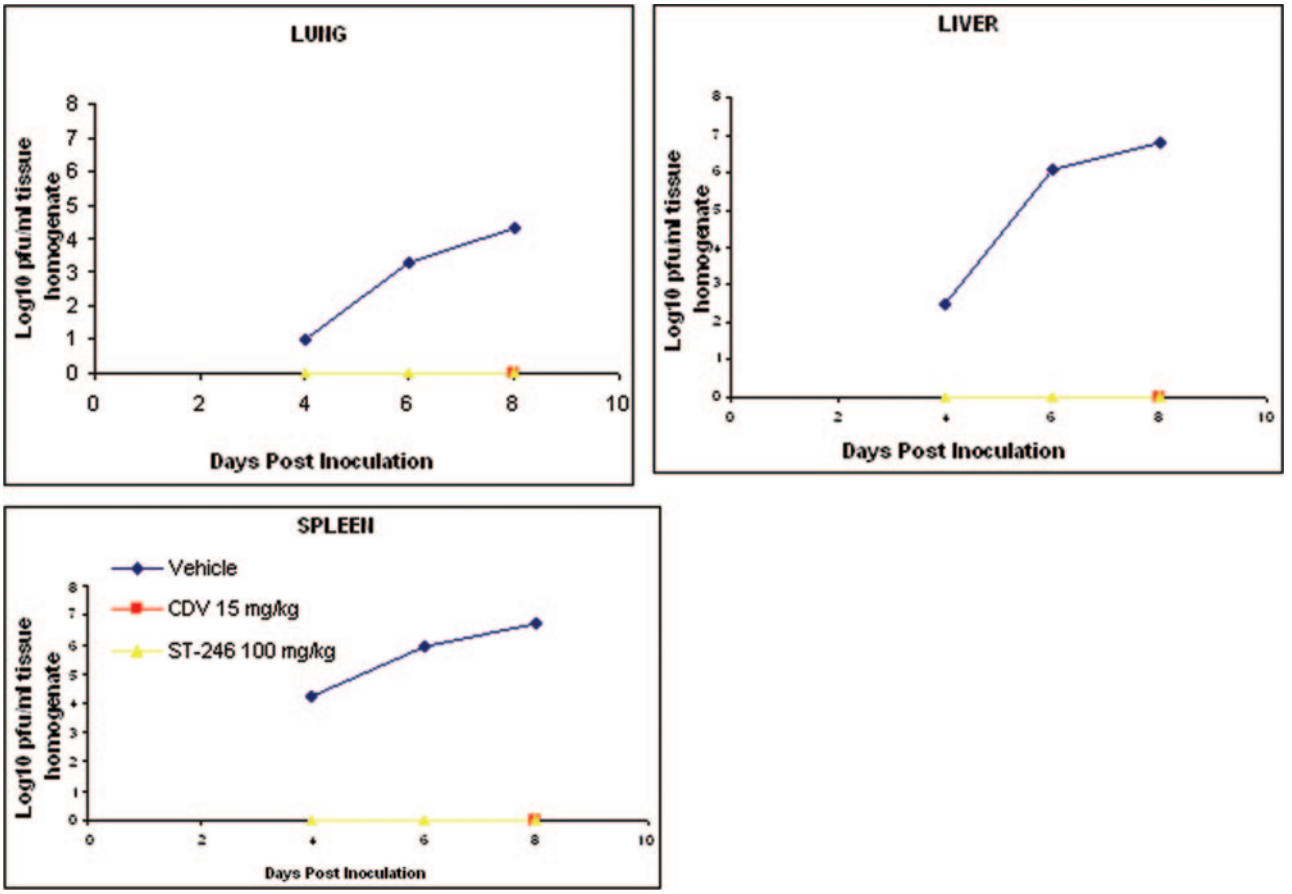

FIG. 3. Effects of once-daily oral dosing with 100 mg/kg ST-246 using a 14-day duration in A/NCr mice infected intranasally with ECTV. Five mice per group were euthanized, and tissue infectivities for liver, spleen, and lung were measured by plaque assay. Points indicate the mean values obtained from five organ samples per time point. The limit of detection is 10 PFU/ml in a 10% tissue homogenate.

mean day of death is approximately 5 to 6 days (6, 7, 8, 9, 10, 11, 12). When CV or ECTV is used, the mean day of death is extended to 9 to 10 days (1, 2, 6, 7, 8, 9, 10, 13). The approximate timing of viral detection in liver or spleen is day 3 to 5 postinoculation for VV or ECTV or day 5 to 7 postinoculation for CV. This difference in systemic distribution may explain the extra duration needed for protection in the CV model. Because the mode of action of ST-246 is to inhibit the extracellular virus formation, which precedes the systemic viremic spread to target organs, dosing is necessary for periods longer than 5 days (13). Cessation of drug at 5 days, prior to these events, allows mice to succumb to CV infection. In ordinary smallpox where deaths occur 20 to 24 days postexposure, it would seem likely that ST-246 would provide reasonable protection for periods longer than those afforded by postexposure vaccination (3).

The combined attributes of oral availability, little or no toxic side effects, and postexposure efficacy against CV, VV, and ECTV make ST-246 an excellent candidate for further development, including human clinical trials of safety and dose escalation studies. Potentially, ST-246 is suited for further investigation for treating adverse reactions to smallpox vaccination or monkeypox disease in humans, as well as stockpiling in the event of a bioweapon release of smallpox by bioterrorists.

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